

Thin-Layer Chromatography of Carbonyl Compounds  
Separation of Aliphatic Carbonyl  
2,4-Dinitrophenylhydrazones  
into Classes

D. P. SCHWARTZ AND O. W. PARKS

*Dairy Products Laboratory,  
Eastern Utilization Research and Development Division,  
Agricultural Research Service, U. S. Department of Agriculture,  
Washington, D.C.*

*Received September 23, 1963*

INTRODUCTION

Separation of aliphatic carbonyl 2,4-dinitrophenylhydrazones into classes has been accomplished by paper chromatography (3) and by adsorption chromatography on closed columns of zinc carbonate (6), and activated magnesia (5). The technique of thin-layer chromatography (TLC) offers an advantage over both paper and closed column techniques in being either faster or more adaptable to the detection of smaller amounts of a compound, or to both of these.

This paper describes a method for the separation of aliphatic monocarbonyl 2,4-dinitrophenylhydrazones into classes on thin layers of magnesia. The classes investigated are the methyl ketones, saturated aldehydes, 2-enals, and 2,4-dienals. The procedure, which is essentially an adaptation of the closed column technique described by Schwartz *et al.* (5), facilitates the classification of as little as 0.1  $\mu\text{g}$  in approximately 1 hour.

MATERIALS AND METHODS

Seasorb 43 (activated magnesia), obtained from Fisher Scientific Co.,<sup>1</sup> Silver Spring, Maryland, and Celite 545 (John-Manville Co.)<sup>1</sup> are sieved separately; the material passing through a 200-mesh screen is utilized.

Standard TLC equipment (Research Specialties Corp., Richmond, California)<sup>1</sup> was employed except for the developing tank. For the latter, an

<sup>1</sup> Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

all-glass American Medical Museum Jar No. 10 was employed. The lid of the jar was greased with a paste made of starch and glycerol (1) which afforded an excellent, long-lasting seal.

For preparation of the plates, 15 g of Seisorb 43 and 6 g of Celite were slurried in 45 ml of 95% ethanol in a 125-ml glass-stoppered Erlenmeyer flask by shaking the flask vigorously by hand for 5 minutes. The slurry was poured immediately into the spreader and spread in the standard manner over five 8 × 8 inch plates. The plates were air-dried for 20 minutes and then dried at 100°C for 16–20 hours. The plates were then stored at room temperature in an air-tight, moisture-free desiccator until needed.

Chromatography was carried out in the direction of application of the slurry. The bottom quarter of an inch of adsorbent was scraped off evenly with a straight edge prior to spotting. The spotted plate was placed in a tank which had been previously equilibrated against the solvent system (chloroform:hexane, 85:15) for a least 16 hours. The solvent was permitted to ascend to the top of the plate (about 1 hour) and the plate removed. A permanent record of the plate can be made satisfactorily by using the procedure of Eisenberg (2).

#### RESULTS AND DISCUSSION

Figure 1 is a photograph showing separation of the four classes. The array of compounds shown on the plate represents as difficult a mixture to resolve into classes as one might expect to encounter in practice. As in the closed column method with magnesia (5), the four classes show different colors on the finished plate which aid greatly in differentiating one class from another. Thus, methyl ketones are grey, saturated aldehydes are tan, 2-enals are rust-red, and 2,4-dienals are lavender. The methyl ketones may appear yellow or yellowish-grey as the solvent ascends the plate, but revert to grey as the solvent evaporates from the finished plate.

Also as in the closed column technique, the last or last two members of the classes behave anomalously, moving into the class immediately following. Thus acetone moves into the saturated aldehyde class, acetaldehyde and formaldehyde move into the 2-enal class, and acrolein and crotonal move into the 2,4-dienal class. Each of these compounds, however, shows its true class color. As shown in Fig. 1, nonadecanone through butanone constitute the methyl ketone class, stearic aldehyde through propanal make up the saturated aldehyde class, acetaldehyde through pent-2-enal comprise the 2-enal class, and crotonal through pent-2,4-dienal make up the 2,4-dienal class.

# CHROMATOGRAPHY OF CARBONYL COMPOUNDS

Seven different lots of Seasorb 43 were examined in an effort to ascertain whether any marked differences existed between lots. Six of these lots gave essentially the same degree of separation of the classes and also in the running time of the solvent. There were small variations between the lots, but in all instances separation of the classes was adequately effected.

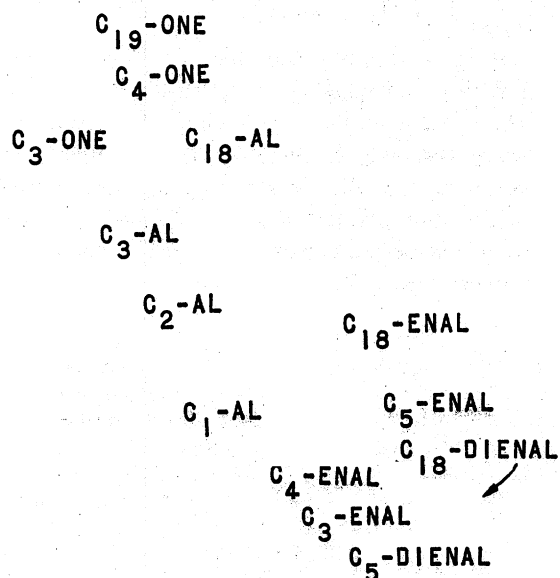


FIG. 1. Photograph of chromatoplate showing separation of the four classes studied. Column at far right represents mixture of all the compounds.

Time of ascent of the solvent varied from about 50 to approximately 70 minutes, with the majority of the lots giving a solvent ascent time of 1 hour. The seventh lot behaved atypically. Separation between the saturated aldehyde, 2-enal, and 2,4-dienal classes was inadequate, but separation of the methyl ketone class from the saturated aldehyde class was excellent and far superior to the separation shown in Fig. 1.

The procedure is inherently sensitive to the detection of micro amounts of 2,4-dinitrophenylhydrazones. It was impossible to readily see the finished spot when  $9 \times 10^{-4}$   $\mu$ moles of a 2,4-dinitrophenylhydrazone from each class was spotted and chromatographed. The method, therefore, may find application as an adjunct to gas chromatographic analysis.

Activated magnesia has seldom been used as an adsorbent for thin-layer chromatography. Kirchner *et al.* (4) used magnesia with a starch binder in their original chromatostrip work. No binder was found to be necessary

with magnesia in the present work. Magnesia appears to have a high affinity toward plain window glass. In fact the magnesia-Celite plates described here adhere to glass so well that it is difficult to even intentionally dislodge the layer by violent contact of the plate with a bench top.

The procedure that has been described has been used successfully in this laboratory for the rapid classification of an unknown 2,4-dinitrophenylhydrazone, for a purity check on cuts obtained from column chromatograms, and for the rapid scanning of a complex mixture obtained from natural sources.

No attempt was made to study the effect of gross overloading, or the effect of a marked disproportionality of constituents on the resolution of the classes. It is to be expected that overloading of the spot or the presence of a marked disproportionality of some of the constituents will give poor resolution of one or more of the classes. In examining unknowns in this laboratory, a concentration gradient is always applied to the plate in order to ascertain the most favorable concentration which should be applied to most adequately effect separation of the classes.

#### SUMMARY

A procedure is described for the separation of the 2,4-dinitrophenylhydrazine derivatives of aliphatic carbonyl compounds into classes by thin-layer chromatography on magnesia-Celite plates. The four classes examined were the methyl ketones, saturated aldehydes, 2-enals, and 2,4-dienals. Each class produces a characteristic color to the plate which aids in its classification. The procedure, which takes about 1 hour, will readily permit the classification of as little as  $9 \times 10^{-4}$   $\mu$ moles of any of the classes studied.

#### REFERENCES

1. BURTON, R. B., ZAFFARONI, A., AND KEUTMANN, E. H., Paper chromatography of steroids. II. Corticosteroids and related compounds. *J. Biol. Chem.* **188**, 763-771 (1951).
2. EISENBERG, F., JR., Rapid method for permanent recording of thin-layer chromatograms. *J. Chromatog.* **9**, 390-391 (1962).
3. GADDIS, A. M., AND ELLIS, R., Paper chromatography of 2,4-dinitrophenylhydrazones. Resolution of 2-alkanone, *n*-alkanal, alk-2-enal, and alk-2,4-dienal derivatives. *Anal. Chem.* **31**, 870-875 (1959).
4. KIRCHNER, J. G., MILLER, J. M., AND KELLER, G. J., Separation and identification of some terpenes by a new chromatographic technique. *Anal. Chem.* **23**, 420-425 (1951).
5. SCHWARTZ, D. P., PARKS, O. W., AND KEENEY, M., Separation of 2,4-dinitrophenylhydrazone derivatives of aliphatic monocarbonyls into classes on magnesia. *Anal. Chem.* **34**, 669-671 (1962).
6. VANDUIN, H., Carbonyl compounds in butter. I. Introduction and method. *Neth. Milk Dairy J.* **12**, 74-80 (1958).